

Research Paper

HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN AFRICAN CATFISH, *Clarias gariepinus* FED WALNUT (*Tetracarpidium conophorum* Mull Arg) LEAF AND ONION (*Allium cepa* Linn) BULB SUPPLEMENTED DIETS

ABSTRACT

Juvenile *Clarias gariepinus* were fed diets containing walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residues at different graded levels: control (0%), OB2 (0.5%), OB3 (1.0%), OB4 (1.5%), OB5 (2.0%), WL6 (0.5%), WL7 (1.0%), WL8 (1.5%) and WL9 (2.0%). After 84 days, Packed Cell Volume (PCV), Haemoglobin (Hb) content, Red Blood Cell (RBC), White Blood Cell (WBC), Haematic indices were measured. Biochemical indices such as total protein, Albumin as well as blood serum, aspartate amino-transferase and alanine amino-transferase were investigated before and after the experiment. Data were analyzed using descriptive statistics and ANOVA at $p = 0.05$. The results obtained showed that packed cell volume, haemoglobin content, red blood cell, white blood cell and haematic indices were not significantly different ($p > 0.05$) among the dietary groups. There were increases in total protein and albumin but values of aspartate amino transferase and alanine amino-transferase decreased though not significantly ($p > 0.05$) among the treatments. However, fish fed the walnut leaf and onion bulb residue-based feeds recorded higher values in PCV, Hb, WBC and total protein compared to the values obtained before experiment and the control. The results of this study suggested that the dietary supplementation of walnut leaf and onion bulb residues affected haematological factors of cultured *Clarias gariepinus* juveniles.

Keywords: Walnut leaf, Onion bulb, Haematology, *Clarias gariepinus*

INTRODUCTION

Aquaculture production has increased tremendously in Nigeria and *Clarias gariepinus* is an economically important cultured fish species. Nutrition is a major factor that determines the physiological well being of an animal. Use of natural feed additives is becoming more important for fish feeding rather than chemical feed additives due to the cumulative effects of the chemical components on animal and human health. Recent studies have shown successful use of spices and natural herbs in fish nutrition including marjoram, licorice roots, black seeds, peppermint, caraway seed, fennel seed and fenugreek seeds (Abdel-Monem *et al.*, 2002; Sakr, 2003; Shalaby *et al.*, 2003; El-Dakar *et al.*, 2004a&b; El-Dakar, 2004; Shalaby, 2004; El-Dakar *et al.*, 2007 and El-Dakar *et al.*, 2008). Also, a number of studies have shown that differences in blood cell formation and function can also be indicative of dietary manipulations.

There is a need to assess the values of locally available spices and herbs such as walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb to determine the nutritional potential of each and the possibility of using them in the commercial fish feed industry. Use of natural herbs, medicinal and aromatic plants in fish feeding is still limited both on the experimental and commercial scales and few studies have been conducted to utilize these herbs and plants as fish feed additives to enhance growth and feed efficiency. This study is aimed at investigating the effects of different levels of inclusion of walnut leaf and onion bulb residues in the diets and haematology of *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

Plant Collection, Identification, Preparation and Extraction:

Onion (*A. cepa*) bulbs were purchased from a market in Ibadan while walnut (*T. conophorum*) leaves were harvested from a farm at Oka -Akoko, Ondo State in Nigeria. They were authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited under FHI 107515.

Onion extraction:

The onion bulbs were washed with distilled water and ~~allowed to air dry~~ at ambient temperature (25°C) for one hour. The dry outer coverings of the onions were manually peeled off, washed and extracted as described by Azu and Onyeagba, (2007). 200g of the fresh onion bulbs were blended into fine pulp and soaked in 100ml of 95% ethanol for 24hrs. It was left in a clean, sterile glass container, shaken vigorously to allow proper extraction and filtered using a sterile muslin cloth. ~~The~~ residue obtained after the removal of the liquid was air-dried and stored at 4°C until required.

Walnut leaf extraction:

The extraction of walnut leaves was as described by Ajaiyeoba and Fadare (2006). The air – dried walnut leaves were ground with a hammer mill to fine powder and 200 g of the powder mixed thoroughly with methanol and filtered using a sterile muslin cloth. The residue obtained after extraction was air – dried and stored at (25°C) until required.

EXPERIMENTAL SYSTEM

The experiment was carried out in twenty seven plastic experimental tanks (50x34x27cm) for 12 weeks in the Fisheries Laboratory, University of Ibadan, Nigeria. The water level in each tank was maintained at volume of 35 litres throughout the study period. Water in each tank was replaced every three (3) days throughout the period to maintain relatively uniform physiochemical parameters and prevent fouling resulting from feed remnants. The source of water was from University of Ibadan water station and each tank was well aerated using ~~air stone and aerator pumps~~ (Lawson, 1995). The mean water temperature, pH and dissolved oxygen were recorded as $26.79 \pm 1.25^{\circ}\text{C}$, 7.83 ± 0.26 and 6.97 ± 0.09 mg/l respectively

EXPERIMENTAL PROCEDURE & FEEDING TRIALS

There were nine experimental diets during this study. Each treatment had three replicates with 20 fish per replicate and mean initial body weight of $7.39 \pm 0.02\text{g}$. The fish were acclimatized for fourteen days before the experiment ~~which lasted for 12 weeks and the fish~~ were fed at 3% body weight daily. ~~The diet per day was divided into two: 1.5% given in the morning by 8.00 – 9.00 am and 1.5% in the evening by 5.00 pm.~~

PREPARATION OF EXPERIMENTAL DIETS

The **mean proximate composition**  the experimental diet was 40.0% ~~crude~~ protein, 15.9% ether extract, 15.7% ash, 7.4% moisture, and 20.9% Nitrogen Free Extract. Nine experimental diets were prepared by incorporating walnut leaf and onion bulb residues at the following inclusion levels; 0 (control), 0.5%, 1.0%, 1.5% and 2.0% respectively. Feed ingredients such as fishmeal, soybean, maize, starch, vegetable oil, ~~Di~~calcium phosphate (DCP), salt and vitamin- mineral premix were added. The dry ingredients were mixed thoroughly in a mixer, water was added and

the resulting dough pelletized. The pellets were ~~diets were~~ sun –dried, and stored in airtight containers at room temperature until required.

HAEMATOLOGICAL AND BIOCHEMICAL ANALYSES

Haematological analysis of the fish was carried out before and after the experiment at the Haematological Laboratory of Veterinary Pathology Department, University of Ibadan within 30 minutes of sampling. 3 – 4cm from the genital opening of each fish was punctured and wiped with dry tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish. The blood was taken under gentle aspiration until about 3ml had been obtained. The needle was gently withdrawn and the blood transferred into heparinized plastic containers and complete haematology was done as described by the methods of **Blaxhall and Daisley (1973)**.

Blood samples for biochemical analysis were centrifuged for 5 minutes at 3000rpm with Hawsley minor bench centrifuge. The derived was stored at -20°C and analyzed at the Haematology Laboratory of Veterinary Pathology Department, University of Ibadan for total protein, albumin, globulin, albumin – globulin ratio and blood serum enzymes such as Aspartate amino transferase (AST) and Alanine amino transferase (ALT) as described by the methods of **Blaxhall and Daisley (1973)**.

STATISTICAL ANALYSIS

Haematology, biochemical indices and blood serum resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 15.0). Duncan multiple range test was used to compare differences among individual means.

RESULTS

HAEMATOLOGY OF *Clarias gariepinus* BEFORE AND AFTER FEEDING TRIALS

The mean values of some haematological parameters of *Clarias gariepinus* was presented on Table 1

Table 1: Mean Values of Some Haematological Parameters of African Catfish *Clarias gariepinus* Juveniles before and after the feeding experiment

Parameters	Before experiment	After feeding experiment								
		CONTROL	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
PCV (%)	12.50±2.50	26.00±4.00 ^{ab}	24.00±1.41 ^{ab}	28.00±2.83 ^{ab}	22.00±0.89 ^{ab}	20.00±1.70 ^a	29.50±2.12 ^{ab}	25.00±4.24 ^{ab}	28.00±1.10 ^{ab}	34.50±0.71 ^b
Hb (gm %)	4.10±0.45	8.30±1.98 ^{ab}	7.25±0.64 ^{ab}	8.80±1.41 ^{ab}	6.70±2.97 ^{ab}	5.65±0.07 ^a	9.35±0.92 ^{ab}	7.70±1.41 ^{ab}	9.10±3.25 ^{ab}	10.65±0.07 ^b
RBC x10 ^{12/L}	1.07±0.05	2.47±1.12 ^a	2.02±0.50 ^a	2.90±0.59 ^a	2.38±1.75 ^a	1.75±0.05 ^a	2.90±0.52 ^a	2.20±0.53 ^a	2.55±1.26 ^a	3.39±0.03 ^a
WBC x10 ^{9/L}	15,250±5.50	11,650±2.10 ^a	16,025±3.64 ^a	17,325±8.24 ^a	14,875±3.14 ^a	13,400±4.10 ^a	17,050±2.68 ^a	16,050±9.68 ^a	13,625±1.87 ^a	13,500±3.82 ^a
Platelet	133,000±1.10	99,500±4.94 ^a	129,000±3.50 ^{ab}	203,000±4.10 ^b	135,500±1.06 ^{ab}	112,000±4.24 ^a	123,000±1.55 ^a	128,500±2.89 ^{ab}	160,000±4.81 ^{ab}	107,000±9.89 ^a
ESR (mm/hr)	0.2±0.14	0.3±0.14 ^b	0.15±0.07 ^{ab}	0.10±0.00 ^a	0.25±0.07 ^{ab}	0.15±0.07 ^{ab}	0.20±0.00 ^{ab}	0.20±0.14 ^{ab}	0.10±0.00 ^a	0.10±0.00 ^a
MCV (fl)	118.17±0.38	111.49±0.62 ^a	122.02±0.33 ^a	97.60±0.70 ^a	95.24±0.75 ^a	114.66±0.23 ^a	102.88±0.60 ^a	113.84±0.13 ^a	112.57±0.40 ^a	101.76±0.68 ^a
MCH (Pg)	3.91±0.86	3.55±0.80 ^a	3.68±0.60 ^a	3.05±0.14 ^a	2.76±0.050 ^a	3.24±0.05 ^a	3.26±0.26 ^a	3.50±0.20 ^a	3.71±0.56 ^a	3.15±0.07 ^a
MCHC (%)	34.00±0.04	32.00±0.00 ^b	31.00±0.01 ^{ab}	32.00±0.02 ^b	31.00±0.01 ^{ab}	29.00±0.01 ^a	32.00±0.04 ^b	31.00±0.01 ^{ab}	33.00±0.01 ^b	31.00±0.00 ^{ab}
Lym (%)	69.00±1.00	66.00±0.07 ^a	68.00±0.00 ^a	66.00±0.07 ^a	67.50±0.71 ^a	67.00±0.41 ^a	67.00±0.65 ^a	68.00±5.66 ^a	70.00±0.07 ^a	68.50±0.49 ^a
Hetero (%)	25.00±2.00	29.50±2.12 ^a	25.00±1.40 ^a	30.00±8.49 ^a	30.00±1.41 ^a	32.00±2.83 ^a	31.00±2.83 ^a	36.00±7.07 ^a	33.50±7.78 ^a	31.00±8.49 ^a
Mono	3.00±0.00	1.50±0.71 ^a	2.50±0.07 ^a	2.00±1.41 ^a	1.50±0.71 ^a	1.50±0.71 ^a	2.50±0.07 ^a	1.50±0.11 ^a	2.00±1.41 ^a	1.50±0.71 ^a
Eos	3.00±1.00	2.50±2.12 ^a	4.50±0.71 ^a	2.00±0.00 ^a	1.00±0.00 ^a	1.50±2.12 ^a	1.50±2.12 ^a	1.50±0.71 ^a	2.50±0.71 ^a	2.50±2.12 ^a

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

NOTE: PCV = packed cell volume, Hb =Haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, ESR = Erythrocytes sedimentation rate, MCV =Mean Cell Volume, MCH = Mean Cell Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, Lym =Lymphocytes, Hetero =Heterophil, Eos = Eosunophil, Mono = Monocytes

PLASMA BIOCHEMISTRY OF *Clarias gariepinus* BEFORE AND AFTER FEEDING TRIALS

The result of plasma biochemistry of *Clarias gariepinus* was presented on Table 2

Table 2: Mean Plasma Biochemistry Parameters of African Catfish *Clarias gariepinus* Juveniles before and after the feeding experiment

Parameters	Before experiment	After feeding experiment								
		CONTROL	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Total protein	3.10±0.14	2.85±0.49 ^a	4.60±0.05 ^{ab}	3.95±1.06 ^{ab}	2.60±0.85 ^a	4.00±0.57 ^{ab}	2.90±0.14 ^a	5.70±0.99 ^b	4.15±0.92 ^{ab}	3.60±1.99 ^{ab}
Albumin	0.95±0.35	2.35±0.21 ^a	2.25±0.07 ^a	2.30±0.00 ^a	1.90± 1.27 ^a	2.60±0.57 ^a	2.50±0.28 ^a	2.75±0.07 ^a	2.45±0.64 ^a	1.95±1.06 ^a
Globulin	2.15±0.49	0.50±0.28 ^a	2.35±0.07 ^{bc}	1.65±1.06 ^{abc}	0.70±0.42 ^a	1.40±0.00 ^{ab}	0.40±0.14 ^a	2.95±1.06 ^c	1.50±0.00 ^{abc}	1.65±0.92 ^{abc}
A.G Ratio	0.50±0.28	0.20±0.14 ^a	1.00±0.00 ^b	0.65±0.49 ^{ab}	0.55±0.64 ^{ab}	0.55±0.07 ^{ab}	0.15±0.07 ^a	1.00±0.42 ^b	0.65±0.21 ^{ab}	0.80±0.00 ^{ab}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

BLOOD SERUM ENZYMES OF *Clarias gariepinus* BEFORE AND AFTER FEEDING TRIALS

The result of the experiment shows that highest value of AST was recorded in OB2 and lowest in WL 8 while highest ALT was recorded in OB3 and lowest in WL 8, these values were lower than the one obtained before the experiment.

Table 3: Blood Serum of African Catfish *Clarias gariepinus* Juveniles before and after the feeding experiment

Parameters	Before experiment	After feeding experiment								
		CONTROL	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
AST(IU/l)	151.00±1.31	132.00±4.24 ^a	136.00±2.83 ^a	141.50±9.19 ^a	135.00±9.90 ^a	131.00±9.89 ^a	131.00±4.14 ^a	139.00±9.90 ^a	129.50±6.26 ^a	137.50±10.61 ^a
ALT(IU/l)	68.00±8.49	24.00±4.14 ^a	22.00±7.07 ^a	26.00±5.66 ^a	19.00±2.83 ^a	20.00±2.83 ^a	20.00±7.07 ^a	21.50±2.12 ^a	18.00±2.83 ^a	22.50±3.84 ^a

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Note: AST =Aspartate amino transferase ALT =Alanine amino transferase

DISCUSSION

As immunological studies continue to include dietary manipulation, haematology becomes a necessary research tool for further interpretation of dietary effects. Examination of the fish before and after the experiment revealed that the fish were in good condition free from disease and infections. However, there was decrease in some haematological and biochemical parameters after the experiment when compared with the ~~values~~ recorded before the experiment. This could be attributed to stress encountered during sampling, capture and handling procedure, which must have increased the catecholamine secretion ~~and production of haemoconcentration in fish~~. The mean PCV(%) values of *Clarias gariepinus* juveniles fed different graded level of onion bulb (OB) and walnut leaf (WL) of 0.5%, 1.0%, 1.5% and 2.0% respectively showed general increase in values when compared with the values obtained before the experiment

This study revealed that ~~supplement~~ of onion bulb and walnut ~~leaves~~ residues in fish feed induced increases in the blood parameters like (erythrocyte count, heamoglobin content and hematocrit values ~~in treated fish~~. From the result obtained in PCV, Hb and RBC during the study showed that the onion bulb and walnut leaf residues inclusion does not result into anaemia and this could support its uses in aquaculture as safe plant immunostimulants. This suggests that walnut leaf and onion bulb residues could enhance non- specific immune responses. The result also, shows that white blood cell recorded in the treated groups were higher in values compared to the control which reflected that fish fed walnut leaf and onion bulb diet were able to build immunity against pathogens. The findings of this study were similar to the results of Shalaby *et al.*, (2006) who reported a significant ($p<0.05$) increase in erythrocyte count, heamoglobin content and hamatocrit value in *O. niloticus* fed

different graded levels of garlic (*Allium sativum*) and chloramphenicol. Also, the results of Martins *et al.*, (2002) showed that addition of *Allium sativum* to fish diets increased erythrocyte number, hemoglobin content, hematocrit value, leucocytes and thrombocytes. Faisal (2003) reported that significantly increased values of erythrocyte count, hemoglobin content and hematocrit in catfish, *Clarias gariepinus* at 1st and 3rd days after administration of ciproflatoxin, amoxicillin and ampicillin.

Also, Azza *et al.*, (2009) recorded that the erythrocyte-count was significantly higher with treated groups than the control. Aly *et al.*, (2008) reported that the hematocrit values were significantly higher in the group fed on diet supplemented with probiotic, when compared with the control group. Das *et al.*, (2009) reported that a significant ($p < 0.05$) increase in WBC and RBC was observed in group fed treated diet of different graded level of *Euglena viridis* compared to the control. Onion bulb (*Allium cepa*) and walnut leaves (*Tetracarpidium conophorum*) have some constituents (alkaloids, flavonoids, tannin and thiosulfinates) that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen and bone marrow.

Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of anaemia in most animals Coles, (1986). There were decreases of MCV, MCH and MCHC in fish fed onion bulb and walnut leaves residue –based diets which is in agreement with the work of Shalaby *et al.*, (2006) who reported decrease of MCV, MCH and MCHC in fish on the highest level of chloramphenicol. The differential leucocytic-count is an indicator of health in fish (Fox *et al.*, 1997). The current study showed insignificant changes in the counts of the large lymphocytes, heterophils and monocytes among the experimental groups. This is in agreement with the results of Shalaby *et al.*, (2006) who reported insignificant change in the count of

the large lymphocytes, heterophils and monocytes among the experimental groups. Cuesta *et al.*, (2005) reported that water and ethanolic-extracts of propolis increased the percentage of phagocytes (monocyte-macrophages and acidophilic granulocytes) of gilthead seabream. ~~The neutrophil count was significantly increased in blood of rainbow trout with infected bacterial or intoxicated with heavy metal (Witesta, 2005).~~

~~However, this result support the work of Das *et al.*, (2009) that the feeding of algal and herbal diets supports the notion of antimicrobial properties of the algae *E. viridis*. Changes in the physiological state often reflect alteration of hematologic and blood biochemical values. Clinical chemical analysis is a fundamental tool used to diagnose and predict the outcome of diseases and to monitor the effects of therapeutic, nutritional and environmental management in human and veterinary medicine (Shalaby *et al.*, 2006).~~

Result obtained from this study shows an increase in total protein, albumin, globulin and albumin – globulin ratio. These findings were similar to results obtained by Sahoo and Mukherjee, (2001) in which total protein levels remained unchanged irrespective of aflatoxin / levamisole exposure, although the increased albumin / globulin ratio noted due to aflatoxin treatment was restored by levamisole feeding. Another study by EL-Boushy and EL-Ashram, (2002) in African catfish reported that levamisole was found to restore the total protein level in immune-compromised fish and increasing γ -globulin levels in healthy fish.

However, Das *et al.*, (2009) reported that the serum total protein after long term ~~feed~~^{feed} with *E. viridis* increased in comparison to the control diet which supported the present findings. Also, Siwicki (1989) observed an increase in total protein content after feeding of β -glucan (0.2%) and chitosan (0.5%) in the diet. Serum albumin and globulin values in fish fed with *E. viridis* were higher than the control.

Wiegertjes *et al.*, (1996) reported that increases in serum protein, albumin and globulin levels are thought to be associated with a stronger innate immune response of fish. Hussein *et al.*, (2001) ~~who~~ showed that serum total protein content was elevated in male Albino rats after administration of garlic oil. ~~Also, Faisal (2003) found that serum total protein increased significantly in *Clarias gariepinus* after administration of both ciprofloxacin and amoxycillin antibiotics.~~ Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Shalaby *et al.*, 2006).

Results of this study showed that serum enzymes Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) activities decreased in the fish group fed on all levels of inclusion of *A. cepa* and *T. conophorum* compared to control. There were no significant differences ($P < 0.05$) among the treatments of AST and ALT. These findings were similar to Shalaby *et al.*, (2006) that serum AST and ALT activities decreased significantly in the fish group fed on all levels of *Allium sativum* and chloramphenicol. This work also agreed with those reported by El-Shater *et al.*, (1997) and Augusti *et al.*, (2001), who found that the lipid parameters and enzyme activities (AST, ALT, and ALP) in serum of rats decreased significantly when they were fed on a diet containing 5% *Allium sativum*. Also, Faisal (2003) mentioned reduced AST in serum of catfish after ampicillin administration. These results can be attributed to *Allium cepa* and *T. conophorum*, which may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damage to the liver cells. This is reflected in the reduction of liver enzymes. *Allium cepa* and *T. conophorum* help the liver to maintain its normal function by accelerating the regenerative capacity of its cells.

In conclusion, this work revealed that the immune response studied in fish can be affected by dietary feed intake. Therefore, dietary supplementation of walnut leaf and onion bulb residues in African catfish, *Clarias gariepinus* feeds should be encouraged since the values obtained were within acceptable limits for cultured fish and no traces of infection such as anaemia were observed in the cultured fish, *Clarias gariepinus*. 

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